bio-protocol Preprint

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Generation of rescued mouse ES cell lines expressing EGFP-tagged Suv39h proteins

TJ Thomas Jenuwein KMS Kalina Malgorzata Swist-Rosowska

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An abbreviated version of this protocol was published in eLIFE in Aug 2017

Major satellite repeat RNA stabilize heterochromatin retention of Suv39h enzymes by RNA-nucleosome association and RNA:DNA hybrid formation

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Detailed protocol

1. Sequences coding for mouse Suv39h1-EGFP, Suv39h2-EGFP and Suv39h2Δ(T3-K81)-EGFP were cloned into the pCAGS-IRES-Puro vector using th XhoI and NotI restriction sites. The plasmids were linearized using the PvuI restriction site.
2. 2 ml of 2% w/v gelatin (G1890, Sigma-Aldrich) in water, was add to each well of a 6-well tissue culture dish (83.3920, Sarstedt) and incubated for 20 min at room temperature.
3. The gelatin solution was removed and 2ml of High Glucose DMEM medium (D6171, Sigma-Aldrich) containing 15% Serum Replacement (10828-028, Gibco), 2 mM L-glutamine (35050-038, Gibco), 1 mM sodium pyruvate (11360-039, Gibco), 0.1 mM beta-mercaptoethanol (21985023, Gibco), 1x non-essential amino acids (M7145, Sigma-Aldrich), 100 U/ml penicillin and 100 µg/ml streptomycin (P0781, Sigma-Aldrich), and 1 ml of homemade Leukemia Inhibitory Factor was add to each well.
4. Mouse *Suv39h dn* ES cells (d57, generated in the Jenuwein Laboratory) were plated in the gelatinized 6-well tissue culture dish at a density of $0.5x10^6$ cells per well and allowed to recover in the tissue culture incubator.
5. The medium was replaced with 1 ml of the fresh medium the next day in preparation for stable. transfection.
6. 5 µg of each plasmid was diluted in Xfect Reaction Buffer (631318, Clonetech) to a final volume of 100 µl and vortexed for 5 s at the highest speed.
7. 1.5 µl of Xfect polymer (631318, Clonetech) was added to each plasmid dilution.
8. Samples were vortexed for 10 s at the highest speed and then incubated at room temperature for 10 min.
9. 100 µl of the DNA-polymer solution was add to each well dropwise.
10. The cells were incubated for 4h at 37°C and the medium was replaced with 2ml of fresh medium.
11. 48h after transfection, the medium was replaced with medium containing 1 µg/ml puromycin (P8833, Sigma-Aldrich).
12. Medium was replaced everyday with fresh medium containing 1 µg/ml puromycin, during antibiotic selection.
13. Cells were maintained in medium containing 1 µg/ml puromycin once cell-lines were established and characterized.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Jenuwein, T. and Swist-Rosowska, K. M.(2023). Generation of rescued mouse ES cell lines expressing EGFP-tagged Suv39h proteins. Bio-protocol Preprint. bio-protocol.org/prep2186.
2. Velazquez Camacho, O., Galan, C., Swist-Rosowska, K., Ching, R., Gamalinda, M., Karabiber, F., De La Rosa-Velazquez, I., Engist, B., Koschorz, B., Shukeir, N., Onishi-Seebacher, M., van de Nobelen, S. and Jenuwein, T.(2017). Major satellite repeat RNA stabilize heterochromatin retention of Suv39h enzymes by RNA-nucleosome association and RNA:DNA hybrid formation. eLIFE. DOI: 10.7554/eLife.25293

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